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Determination of Flavonoid Content in Tempuyung Leaf Extract (*Sonchus arvensis* L.) across Different Fractions

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Abstract

Tempuyung (*Sonchus arvensis* L.) leaves are traditionally used as medicinal material and contain flavonoid compounds whose recovery may vary according to solvent polarity during extraction and fractionation. This study determines the total flavonoid content of the aqueous, ethyl acetate, and n-hexane fractions of *S. arvensis* leaf extract and identifies the fraction containing the highest concentration. A descriptive observational method was employed. The leaves were extracted by maceration with 96% ethanol, followed by liquid–liquid fractionation using distilled water, ethyl acetate, and n-hexane. Flavonoids were qualitatively identified using Mg-HCl and $FeCl_3$ reagents, while total flavonoid content was quantified by UV–Vis spectrophotometry using quercetin as the reference standard at a maximum wavelength of 421 nm. Qualitative screening confirmed the presence of flavonoids in the crude extract and all fractions. The aqueous, ethyl acetate, and n-hexane fractions contained 17.111 mg quercetin equivalents per gram (mg QE/g; 1.7111%), 91.887 mg QE/g (9.1887%), and 7.9333 mg QE/g (0.7933%), respectively. The ethyl acetate fraction therefore exhibited the highest total flavonoid content, followed by the aqueous and n-hexane fractions. These findings demonstrate that solvent polarity influences the distribution of flavonoids during fractionation and identify ethyl acetate as the most effective of the examined solvents for obtaining a flavonoid-enriched fraction of *S. arvensis* leaf extract. The study provides a practical basis for

selecting an appropriate fraction for subsequent phytochemical and pharmacological investigation.

Keywords: Ethyl Acetate Fraction; Fractionation; *Sonchus arvensis* L.; Total Flavonoid Content; UV–Visible Spectrophotometry

INTRODUCTION

Indonesia is one of the tropical countries with the second-highest diversity of plant species after Brazil. This is reflected in the wide variety of plants found in Indonesia, each possessing distinctive characteristics in terms of roots, stems, leaves, and fruits. Traditionally, Indonesian communities have utilized plants to treat various diseases. One plant that has been effectively used for disease prevention is tempuyung (*Sonchus arvensis* L.) leaves (Rahayu et al., 2021). *Sonchus arvensis* L., widely known as tempuyung, belongs to the family Asteraceae and the genus *Sonchus* (Gunarti et al., 2021). The ability of tempuyung (*Sonchus arvensis* L.) leaves to reduce uric acid levels has been empirically demonstrated (Adelia, 2020).

Tempuyung leaves contain various mineral compounds, including potassium, silica, sodium, and magnesium, as well as several flavonoids, such as kaempferol, luteolin-7-O-glucose, and apigenin-7-O-glucose; coumarin (scopoletin); taraxasterol; inositol; and phenolic acids, such as cinnamic, coumaric, and vanillic acids. Studies have shown that the flavonoid compounds in tempuyung leaves have the ability to reduce uric acid levels by inhibiting the activity of the xanthine oxidase enzyme, which converts xanthine into uric acid (Adelia, 2020; Gultom, 2023).

Uric acid is classified as a non-communicable disease that may arise from excessive consumption of purine-rich foods (Alawaadh et al., 2020). An imbalance between uric acid intake and excretion can lead to the deposition of uric acid crystals in body tissues (Kurniawan et a;l., 2018). Uric acid is the final product of purine metabolism and is a constituent of nucleic acids present in the body. The accumulation of crystals in the joints is associated with increased purine levels (Songgigilan et al., 2019), which may increase blood uric acid concentrations by approximately 0.5–0.75 g/mL for every gram of purine consumed. Research conducted by the World Health Organization indicates that Indonesia has one of the highest incidences of gout globally. Findings from the WHO–International League of Associations for Rheumatology Community Oriented Program for Control of Rheumatic Disease (WHO-ILAR COPCORD) in rural areas of North Sulawesi and Manado

revealed an association between chronic uric acid levels and dietary practices, including alcohol consumption and the consumption of purine-rich foods such as seafood. High alcohol consumption and a protein-rich diet, particularly from shellfish and various fish species, significantly increase the risk of developing gouty arthritis (Singh et al., 2019).

The prevalence of hyperuricemia in Indonesia is approximately 15% (Santoso et al., 2021). Based on data published by the World Health Organization (WHO) in 2017, 34.2% of the world's population suffered from gout. According to estimates by the World Health Organization (WHO), the number of deaths attributable to gout is expected to increase by up to 55% by 2060 (Mattiuzzi & Lippi, 2019). In 2018, a study conducted by the Basic Health Research (Riset Kesehatan Dasar; Riskesdas) reported that 713.783 million people (7.30%) in Indonesia suffered from joint diseases. Joint diseases are more prevalent among older age groups, with frequencies of 15.55% (79.919 million) among individuals aged 55–64 years, 18.63% (38.572 million) among those aged 65–74 years, and 18.95% (17.822 million) among individuals aged over 75 years.

Tempuyung leaves have benefits in reducing uric acid levels. This effect is attributed to their active flavonoid compounds, which function as natural antioxidants. These compounds can help reduce the production of the xanthine oxidase enzyme, which has the potential to act as an antihyperuricemic inhibitor. In addition, tempuyung leaves are beneficial in reducing uric acid levels and dissolving urate crystals. Joint pain commonly experienced by individuals with gout is caused by the accumulation of urate crystals in the blood vessels. Regular consumption of tempuyung leaves may also prevent the formation of enzymes that can increase uric acid levels (Rana & Gulliya, 2019).

The predominant compounds present in tempuyung leaves are flavonoids, a class of flavone compounds that function as antioxidants. Flavonoids act by inhibiting the activity of xanthine oxidase and superoxide, thereby inhibiting the formation of uric acid. One type of flavonoid that is thought to have an effect on reducing uric acid levels is apigenin 7-O-glucoside (Adelia, 2020).

Flavonoids are one of the most abundant groups of secondary metabolites found in plant tissues (Sholehah & Setiawan, 2019). Flavonoids are polar chemical compounds containing sugar moieties or hydroxyl groups that are soluble in polar solvents, including ethyl acetate, butanol, methanol, and ethanol, as well as dimethyl sulfoxide, dimethylformamide, and water. This polarity enables flavonoids to exhibit various biological

effects, including immunomodulatory, hypolipidemic, and hypoglycemic activities, as well as the ability to promote blood vessel relaxation and function as antioxidants. Flavonoids are also effective in inhibiting oxidation reactions through both enzymatic and non-enzymatic mechanisms and act as free-radical scavengers, thereby protecting membrane lipids from potential damage (Satria et al., 2022).

According to research conducted by Harahap, (2019) the results met the required criteria, indicating a moisture content of 5.33%. The minimum solubility in water was 28.27%, whereas the maximum solubility in ethanol was 9.42%. The reported total ash content was 16.44%, while the acid-insoluble ash content was relatively high at 0.61%. In addition, phytochemical screening of tempuyung leaves revealed the presence of flavonoids, alkaloids, tannins, anthraquinones, glycosides, steroids/terpenoids, and saponins. Furthermore, research conducted by Artini & Aryasa, (2019) demonstrated that serum uric acid levels in potassium-oxonate-induced white rats (*Rattus norvegicus*) could be effectively reduced by an infusion of tempuyung roots (*Sonchus arvensis*) at doses of 1.25 g/kg body weight (10% concentration), 2.5 g/kg body weight (20% concentration), and 5 g/kg body weight (40% concentration). Meanwhile, research by Sari, (2020) demonstrated that the total flavonoid content of acid-hydrolyzed tempuyung extract was 0.532%, whereas the total flavonoid content of non-hydrolyzed tempuyung leaf extract was 0.388%.

The presence of flavonoids alone is considered insufficient to fully characterize the potential of a natural material. Therefore, determination of the total flavonoid content is necessary to obtain quantitative information on the flavonoid concentration in tempuyung leaf extract. This information is important because the content of bioactive compounds in plants can be influenced by the extraction process and the characteristics of the solvents used. Flavonoids are one of the major groups of secondary metabolites widely distributed in plant tissues and generally exhibit polar characteristics due to the presence of hydroxyl groups and sugar moieties. These differences in polarity allow flavonoid compounds to be distributed differently during the separation process when solvents with varying degrees of polarity are employed.

Fractionation is a process of separating compounds based on the use of two immiscible solvents. One of the advantages of this technique is that it can be readily automated, allowing large quantities of samples to be processed efficiently. In this study, three fractions were used, namely n-hexane, which is non-polar; ethyl acetate, which is semi-

polar; and distilled water (aquadest), which is polar, to compare flavonoid content using the spectrophotometric method. One advantage of the spectrophotometric method is the simplicity of the equipment required to identify elements, even at very low concentrations. In general, the upper detection limit of this method involves the measurement of elemental concentrations below 1–2% or even lower, such as micrograms per liter. Another advantage of this method is its ease of automation, allowing large numbers of samples to be processed automatically within a short period (Syifa *et al.*, 2022).

UV–Vis (Ultraviolet–Visible) spectrophotometry is an analytical technique commonly used in studies of chemical compounds. UV–Vis spectrophotometric measurements focus on the absorption of light within specific wavelength ranges, namely 200–350 nm for ultraviolet light and 350–800 nm for visible light, as the light interacts with specific compounds (Mayang & Santoso, 2020). The determination of total flavonoid content frequently employs UV–Vis spectrophotometry because flavonoids contain conjugated aromatic groups that enable them to efficiently absorb light in both the ultraviolet and visible spectra. With the addition of reagents such as aluminum chloride ($AlCl_3$), flavonoids can be quantitatively analyzed using a UV–Vis spectrophotometer, with quercetin commonly used as a reference standard for measurement. Quercetin is a flavonoid that is widely distributed in plants and constitutes approximately 60–75% of all flavonoids, including glycosides. It is capable of forming a complex with $AlCl_3$ and belongs to the flavonol subclass of flavonoids after reacting with it (Kencanawati, 2022).

The novelty of this study lies in the determination and comparison of the total flavonoid content of tempuyung leaf extract following fractionation based on solvent polarity, particularly in the aqueous, ethyl acetate, and n-hexane fractions. This approach is expected to provide more specific information regarding the fraction with the highest total flavonoid content compared with previous studies that analyzed the extract without fractionation. In addition, this study employs UV–Vis spectrophotometry to determine the total flavonoid content, using quercetin as the reference standard. UV–Vis spectrophotometry was selected because flavonoids possess conjugated aromatic systems that enable absorption in both the ultraviolet and visible regions of the spectrum. The addition of $AlCl_3$ facilitates the formation of complexes with flavonoid compounds, thereby enabling their quantitative determination.

Based on the foregoing discussion, the study entitled "Determination of Flavonoid Content in Tempuyung (*Sonchus arvensis* L.) Leaf Extract at Different Fractionation Levels" was conducted to obtain quantitative information on the total flavonoid content of each fraction. The study focused on determining the total flavonoid content of the aqueous, ethyl acetate, and n-hexane fractions of tempuyung leaf extract and comparing the flavonoid contents among the three fractions. The objective of this study was to determine the flavonoid content of the aqueous, ethyl acetate, and n-hexane fractions of tempuyung (*Sonchus arvensis* L.) leaf extract and thereby identify the fraction with the highest total flavonoid content.

METHODS

This study employed a descriptive observational design to determine the total flavonoid content of tempuyung (*Sonchus arvensis* L.) leaf extract in the aquadest, ethyl acetate, and n-hexane fractions. The study was conducted from November 2023 to June 2024 at the Laboratory of Pharmaceutical Chemistry and Technology, Faculty of Health, Sari Mulia University, Banjarmasin. The study population consisted of tempuyung plants grown and cultivated in South Kalimantan, while the sample consisted of tempuyung leaves obtained from Banjarmasin. A total of 600 g of fresh leaves were processed into 400 g of dried crude drug (*simplisia*), after which 250 g of the *simplisia* powder was extracted using 96% ethanol by maceration for 1 × 24 h, followed by three successive remaceration cycles. The resulting extract was fractionated by liquid–liquid partitioning using aquadest, ethyl acetate, and n-hexane. Qualitative analysis of flavonoids was performed using colorimetric tests with Mg-HCl powder and $FeCl_3$, whereas quantitative analysis was conducted using UV–Vis spectrophotometry with quercetin as the standard. The maximum wavelength was determined at 421 nm, and the operating time was established at 22 min. A total of 100 mg of each extract/fraction was dissolved in pro analysis ethanol to a final volume of 100 mL, followed by reaction with 10% $AlCl_3$ and 5% acetic acid, and the absorbance was measured in triplicate. The total flavonoid content was calculated based on the linear regression equation of the quercetin calibration curve and expressed as mg QE/g, followed by descriptive comparison among the fractions.

RESULTS

1. Extraction and Fractionation Results

The tempuyung leaves (*Sonchus arvensis* L.) used in this study were first processed into crude drug (*simplisia*) and extracted using 96% ethanol through the maceration method. The resulting extract was subsequently fractionated based on differences in solvent polarity into aquadest, ethyl acetate, and n-hexane fractions. The calculation results showed that the yield of the 96% ethanol extract was 8.23%. Following the fractionation process, the highest yield was obtained from the aquadest fraction at 36.3%, followed by the n-hexane fraction at 19.6%, while the ethyl acetate fraction produced a yield of 1.9%.

Table 1. Yield of Tempuyung Leaf Extract and Fractions

Sample	Yield (%)
96% ethanol extract	8.23
Aquadest fraction	36.30
Ethyl acetate fraction	1.90
n-Hexane fraction	19.60

Based on the results presented in Table 1, the aquadest fraction produced the highest yield, whereas the ethyl acetate fraction produced the lowest yield. The differences in yield indicate variations in the amounts of components dissolved in each solvent.

2. Results of Qualitative Flavonoid Identification

Flavonoid identification was performed using two color reagents, namely Mg powder + concentrated HCl and $FeCl_3$. A positive result with the Mg-HCl reagent was indicated by the formation of a yellow, orange, or red color, whereas a positive result with $FeCl_3$ was indicated by the formation of a dark green color.

Table 2. Results of the Qualitative Flavonoid Test

Sample	Mg + Concentrated HCl	$FeCl_3$	Result
96% ethanol extract	Red	Dark green	Positive
Aquadest fraction	Orange	Dark green	Positive
Ethyl acetate fraction	Red	Dark green	Positive
n-Hexane fraction	Dark red	Dark green	Positive

The test results on table 2 showed that the 96% ethanol extract and all fractions, namely the aquadest, ethyl acetate, and n-hexane fractions, tested positive for the presence of flavonoids. In the Mg-HCl test, the ethanol extract produced a red color, the aquadest fraction produced an orange color, the ethyl acetate fraction produced a red color, and the n-hexane fraction produced a dark red color. All samples also exhibited a dark green color following the addition of $FeCl_3$.

Thus, no negative results were observed in the qualitative identification of flavonoids among all tested samples.

3. Determination of the Maximum Wavelength and Operating Time

The maximum wavelength was determined using a 100 ppm quercetin standard solution over a wavelength range of 370–450 nm. The measurement results showed that the maximum wavelength of quercetin was obtained at 421 nm.

Table 3. UV–Vis Spectrophotometric Analysis Parameters

Parameter	Result
Standard solution	100 ppm quercetin
Wavelength range	370–450 nm
Maximum wavelength	421 nm
Observation period	60 min
Measurement interval	2 min
Operating time	22 min
Absorbance at operating time	0.936

Subsequently, the *operating time* was determined by measuring the absorbance of the 100 ppm quercetin solution at a wavelength of 421 nm for 60 min, with measurements taken at 2-min intervals. The results showed that the *operating time* was reached at 22 min, with an absorbance of 0.936.

4. Quercetin Calibration Curve Results

The calibration curve was constructed using quercetin standard solutions at concentrations ranging from 10 to 80 ppm. Each concentration was measured in triplicate. The measurement results showed an increase in absorbance with increasing quercetin concentration.

Table 4. Absorbance of Quercetin Standard Solutions

Concentration (ppm)	Mean Absorbance
10	0,084
20	0.152
30	0.226
40	0.317
50	0.445
60	0.552
70	0.658
80	0.799

Based on table 4, the linear regression equation obtained was y = 0.009895x − 0.036578, with an R^2 value of 0.988246. The research file also reported a correlation coefficient of r = 0.9882, indicating a strong linear relationship between quercetin concentration and absorbance.

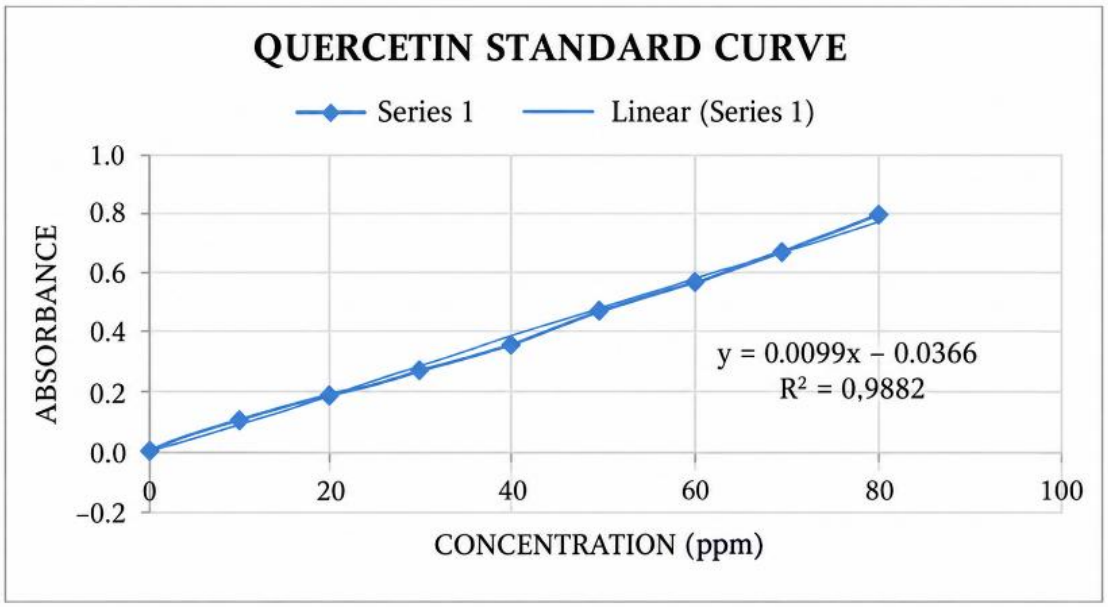

Figure 1. Quercetin Calibration Curve

5. Results of Total Flavonoid Content Determination

The total flavonoid content was determined using UV–Vis spectrophotometry at a wavelength of 421 nm. Each sample was measured in triplicate. The highest mean absorbance value was obtained for the ethyl acetate fraction (0.791), followed by the n-hexane fraction (0.678), 96% ethanol extract (0.424), and aqueous fraction (0.118).

Table 5. Absorbance Measurement Results of the Samples

Sample	Replicate I	Replicate II	Replicate III	Mean
96% Ethanol extract	0.424	0.425	0.425	0.424
Aqueous fraction	0.117	0.118	0.119	0.118
Ethyl acetate fraction	0.787	0.792	0.795	0.791
n-Hexane fraction	0.676	0.679	0.680	0.678

Based on the regression equation of the quercetin calibration curve, the quercetin-equivalent concentrations were determined to be 51.11 ppm for the 96% ethanol extract, 17.11 ppm for the aqueous fraction, 91.88 ppm for the ethyl acetate fraction, and 79.33 ppm for the n-hexane fraction.

Table 6. Total Flavonoid Content of Tempuyung Leaf Extract and Fractions

Sample	Concentration (ppm)	Flavonoid Content (mg QE/g)	Total Flavonoid Content (%)
96% Ethanol extract	51.11	51.11	5.11
Aqueous fraction	17.11	17.11	1.71
Ethyl acetate fraction	91.88	91.88	9.18
n-Hexane fraction	79.33	79.33	7.93

The results showed that in table 6, the ethyl acetate fraction had the highest total flavonoid content, at 91.88 mg QE/g (9.18%), followed by the n-hexane fraction at 79.33 mg QE/g (7.93%), the 96% ethanol extract at 51.11 mg QE/g (5.11%), and the aqueous fraction at 17.11 mg QE/g (1.71%).

Overall, the findings of this study revealed three main results. First, all extracts and fractions tested were positive for flavonoids, as indicated by the Mg–HCl and $FeCl_3$ colorimetric tests. Second, the spectrophotometric analysis demonstrated differences in total flavonoid content among the fractions. Third, the ethyl acetate fraction exhibited the highest total flavonoid content, reaching 91.88 mg QE/g or 9.18%, whereas the aqueous fraction showed the lowest content, at 17.11 mg QE/g or 1.71%.

DISCUSSION

The plant material used in this study consisted of tempuyung leaves (*Sonchus arvensis* L.), which were collected directly from the plant. The processing of the tempuyung leaves began with the collection of the plant material by picking the leaves directly from the plant, followed by wet sorting to remove damaged leaves that were unsuitable for use. The leaves were subsequently washed thoroughly under running water to remove impurities. After washing, the leaves were cut into smaller pieces to facilitate the drying process. The material was dried under direct sunlight until completely dry. Dry sorting was then performed to remove impurities adhering to the material during the drying process, after which the dried leaves were ground using a blender to obtain a fine powder. The sample was dried prior to extraction. The resulting crude drug (simplisia) was subsequently extracted to obtain the chemical constituents present in the plant material. Maceration was selected as the extraction method because it is a simple extraction technique and is capable of extracting heat-sensitive compounds, such as flavonoids (Mohamed & Loumerem, 2024).

The extraction process in this study employed maceration using 96% ethanol as a polar solvent. The maceration process was conducted for 24 h and repeated three times to maximize the extraction of polar flavonoid compounds. The primary objective of applying the maceration method was to optimize extraction efficiency and obtain a more concentrated extract. During maceration, the extracting solvent penetrates the cell walls and enters the intracellular spaces containing active compounds. As the solvent extracts the active constituents, the resulting solution becomes highly concentrated and is subsequently released from the cells due to the concentration gradient between the active-compound solution inside and outside the cells (Asmorowati & Lindawati, 2019). Following maceration, the extract was concentrated using a water bath. The concentration process was intended to evaporate the 96% ethanol solvent and produce a concentrated extract (Dalming et al., 2022).

The yield obtained from the fractionation of plant materials depends on the type of solvent used to extract the constituents of the plant. An extraction yield is generally considered favorable when its value exceeds 10%. Based on the yield calculations, the yield of the tempuyung leaf extract was 8.23%, while the yields of the ethyl acetate, n-hexane, and aqueous fractions were 1.9%, 19.6%, and 36.3%, respectively. These results indicate that the aqueous fraction produced the highest yield compared with the other fractions. Differences in solvent type can affect the amount of extract obtained because solvents differ in their ability to dissolve and extract chemical constituents from plant materials.

The aqueous, ethyl acetate, and n-hexane fractions were subsequently subjected to qualitative analysis for flavonoid compounds. The first identification test was performed by adding magnesium (Mg) powder and concentrated hydrochloric acid (HCl). A positive result is indicated by the development of yellow, orange, or red coloration (Oktavia & Sutoyo, 2021). The identification results showed positive reactions for flavonoids in all fractions, with the n-hexane fraction producing a dark red color, the ethyl acetate fraction producing a red color, and the aqueous fraction producing an orange color. The second identification test involved the addition of ferric chloride ($FeCl_3$), for which a positive result is indicated by the formation of a dark green coloration (Trinovita et al., 2019). In this test, the aqueous, ethyl acetate, and n-hexane fractions all exhibited a dark green coloration, indicating the presence of flavonoid compounds.

The concentrated extracts obtained from each fraction were subsequently subjected to quantitative analysis to determine their total flavonoid content using a UV–Vis

spectrophotometer. Quercetin was used as the reference standard because it belongs to the flavonol subclass of flavonoids and contains a keto group at C-4 and hydroxyl groups at C-3 and/or C-5, which are characteristic structural features associated with flavones and flavonols. When $AlCl_3$ is added to the sample, a complex is formed between aluminum chloride and quercetin, resulting in a bathochromic shift toward the visible region, which is generally manifested as a more intense yellow coloration of the solution. The addition of acetic acid is intended to maintain the wavelength within the visible region (Asmorowati & Lindawati, 2019).

Acetic acid was also added to maintain the stability of the complex formed during the reaction. The preparation of a calibration curve using the standard solution was intended to establish the relationship between solution concentration and absorbance, thereby enabling the concentration of the sample to be determined using a graphical approach (Wardani, 2022). Total flavonoid content was determined using UV–Vis spectrophotometry because flavonoids possess conjugated aromatic systems that produce strong absorption bands within the ultraviolet and visible regions of the spectrum. Absorbance values were used as the basis for quantitative analysis. According to the Lambert–Beer law, absorbance has a linear relationship with the concentration of the analyte; thus, a higher absorbance value indicates a higher concentration of flavonoid compounds in the sample. The quantitative analysis also involved the determination of the operating time, as described by Asmorowati and Lindawati, to establish the period during which the measurement remained stable and the sample reacted optimally to form the desired complex (Asmorowati, 2019). During the 60-min period, measurements were performed at 2-min intervals using a 100 ppm quercetin standard solution for the operating-time determination. Based on the obtained data, a stable response was achieved at the 22nd minute.

The maximum wavelength represents the wavelength at which a compound exhibits its highest absorbance. According to Asmorowati and Lindawati, determination of the maximum wavelength is important for identifying the absorption peak and maintaining a consistent absorbance response. In this study, the maximum wavelength of quercetin was determined by measuring the absorbance of a 100 ppm quercetin standard solution over a wavelength range of approximately 370–450 nm (Asmorowati & Lindawati, 2019). The results showed that the maximum absorption wavelength of the quercetin standard solution was 421 nm, with an absorbance of 0.944. Aminah et al., 2017 reported that quercetin is

commonly selected as a standard because it belongs to the flavonol group of flavonoids, characterized by a keto group at C-4 and hydroxyl groups at C-3 and/or C-5. Quercetin is also widely used as a reference standard for determining flavonoid concentrations. The calibration curve generated from the quercetin standard solution produced the linear regression equation $y = 0.009x - 0.0366$, with a correlation coefficient (r) of 0.9882. The concentrations obtained from the quercetin standard curve showed a direct relationship with absorbance values, indicating that an increase in quercetin concentration corresponded to an increase in absorbance. Quercetin was selected in this study because of its antioxidant properties and its classification as a flavonol, making it a commonly used standard for comparative analysis of flavonoid compounds.

The determination of the maximum wavelength was performed to identify the wavelength corresponding to the maximum absorption peak while maintaining a relatively stable absorbance response. To determine the maximum wavelength of quercetin, the absorbance of a 1000 ppm quercetin standard solution was measured over a wavelength range of 370–450 nm (Asmorowati, 2019). The measurement results indicated that the maximum wavelength was 421 nm. Subsequently, a standard calibration curve was prepared using quercetin standard solutions at different concentrations. The mean absorbance values were 0.084, 0.152, 0.226, 0.317, 0.445, 0.552, 0.658, and 0.799 for concentrations of 10, 20, 30, 40, 50, 60, 70, and 80 ppm, respectively. Absorbance measurements were performed at the maximum wavelength of 421 nm over a 60-min period. The data obtained from the different quercetin standard concentrations demonstrated a linear relationship between concentration and absorbance, indicating that an increase in the concentration of the quercetin standard solution corresponded to an increase in the measured absorbance. Linear regression analysis yielded the equation $y = 0.009x - 0.0366$, with a correlation coefficient (r) of 0.9882. As stated by Asmorowati and Lindawati (2019), a correlation coefficient approaching 1 indicates a linear regression relationship, thereby confirming a strong correlation between absorbance and concentration.

The total flavonoid content of tempuyung leaves (*Sonchus arvensis* L.) was determined following sample preparation, with each sample measured in triplicate. The results showed that the aqueous, ethyl acetate, and n-hexane fractions of tempuyung leaves contained flavonoid compounds, with total flavonoid contents of 17.11 mg QE/g or 1.71% for the aqueous fraction, 91.88 mg QE/g or 9.18% for the ethyl acetate fraction, and 7.93 mg QE/g or 7.9333% for the n-hexane fraction.

The results indicate that the extract and fractions of tempuyung leaves contain various types of flavonoids with different polarities, which may account for the differences in total flavonoid content among the fractions. The concept of "like dissolves like" states that nonpolar compounds tend to dissolve more readily in nonpolar solvents, whereas polar compounds preferentially dissolve in polar solvents. Similarly, semipolar compounds are more readily soluble in semipolar solvents (Syarifuddin & Yusriyani, 2021).

The findings demonstrate that tempuyung leaves (*Sonchus arvensis* L.) contain flavonoid compounds that can be extracted using solvents with different degrees of polarity, with the ethyl acetate fraction exhibiting the highest total flavonoid content. This finding confirms the presence of flavonoids in the extract and fractions, followed by the n-hexane and aqueous fractions in terms of their respective total flavonoid contents.

Three different fractions, namely the aqueous, ethyl acetate, and n-hexane fractions, were compared in terms of their total flavonoid concentrations. According to Kurniawati et al., (2024), ethyl acetate, owing to its semipolar properties, can extract both relatively polar and nonpolar flavonoid molecules. The relatively high abundance of certain free flavonoids in plants, including flavones, flavanones, and flavonols, which are more soluble in semipolar solvents, may account for this finding. In contrast, the nonpolar n-hexane fraction exhibited a lower total flavonoid concentration. Certain flavonoid compounds may dissolve in nonpolar solvents, particularly polymethoxylated aglycones and isoflavones that have been released from their glycosidic forms or possess sugar groups that affect their solubility characteristics. Such compounds may exhibit solubility in nonpolar solvents such as n-hexane.

The present findings are also supported by a previous study conducted by Sari (2020), which investigated the determination of parameters and total flavonoid content in the same plant without applying fractionation. The study reported a total flavonoid content of 0.532% in acid-hydrolyzed tempuyung extract and 0.388% in non-hydrolyzed tempuyung extract. The values obtained in the present study were considerably higher than those reported in the previous study. As previously described, the ethyl acetate fraction of the tempuyung extract exhibited the highest total flavonoid content, reaching 9.18%. This difference may be attributed to the fractionation process applied in the present study, which enabled the separation and partial purification of flavonoid compounds from other constituents that could potentially interfere with the measurement. In the study by Sari (2020), the non-

fractionated tempuyung extract showed a lower total flavonoid content, suggesting that fractionation using specific solvents, particularly ethyl acetate, may substantially increase the concentration of the target bioactive compounds. Therefore, the application of fractionation techniques in the processing of medicinal plant extracts, such as tempuyung, may represent an effective strategy for enriching total flavonoid content and potentially enhancing the pharmacological potential of the resulting extract.

A comparison with the total flavonoid content reported for another plant species further supports these findings. A study conducted by Hesty Wulandari et al. (2022) showed that qualitative analysis of flavonoid compounds using color reactions and thin-layer chromatography (TLC) yielded positive results for the presence of flavonoids. Quantitative analysis using UV–Vis spectrophotometry showed total flavonoid contents of 31.66 mg QE/g or 3.16% in the 96% ethanol extract, 5.16 mg QE/g or 0.51% in the n-hexane fraction, 51.83 mg QE/g or 5.18% in the ethyl acetate fraction, and 7.16 mg QE/g or 0.71% in the methanol fraction. The highest total flavonoid content was observed in the ethyl acetate fraction. This finding is consistent with the results of the present study, in which the ethyl acetate fraction also exhibited the highest total flavonoid content, reaching 91.88 mg QE/g.

The present study also provides further evidence that tempuyung leaves contain flavonoid compounds, consistent with the findings of Harahap, (2019) who previously conducted screening and characterization of tempuyung leaf crude drug (*Sonchus arvensis* L.). The study reported a moisture content of 5.33%, a minimum water-soluble extractive value of 28.27%, an ethanol-soluble extractive value of 9.42%, a total ash content of 16.44%, and an acid-insoluble ash content of 0.61%. Phytochemical screening of tempuyung leaves revealed the presence of flavonoids, alkaloids, tannins, anthraquinones, glycosides, steroids/terpenoids, and saponins.

These findings indicate that flavonoid compounds in tempuyung leaves are relatively more soluble in semipolar solvents, as demonstrated by the highest total flavonoid content obtained from the ethyl acetate fraction.

The findings demonstrate that fractionation provides more specific information regarding the distribution of flavonoids in tempuyung leaf extract. The ethyl acetate fraction exhibited the highest total flavonoid content and may therefore be considered a more promising fraction for further investigations of flavonoid compounds in tempuyung leaves.

These findings further indicate that solvent selection is an important factor in obtaining fractions enriched with specific secondary metabolites.

Scientifically, this study provides additional information to complement previous research that determined the flavonoid content of tempuyung leaf extract without separating the extract according to solvent polarity. Thus, the present study expands the understanding of flavonoid distribution according to solvent polarity, particularly in the aqueous, ethyl acetate, and n-hexane fractions.

These findings also have implications for natural product-based pharmaceutical research, particularly during the preliminary stage of selecting fractions with potentially higher flavonoid content. However, the total flavonoid content determined in this study cannot be used to identify specific flavonoid compounds or directly demonstrate their pharmacological activities. Therefore, the findings should be regarded primarily as preliminary data to support further compound characterization and bioactivity studies.

This study has several limitations, as the analysis was restricted to the determination of total flavonoid content and did not extend to the identification or purification of individual flavonoid compounds present in each fraction. Consequently, the specific flavonoid compounds that are predominant in the ethyl acetate, n-hexane, and aqueous fractions could not be determined. This limitation was also acknowledged in the study, which highlighted the need for further purification and identification to characterize the types of flavonoids present in the samples.

Furthermore, this study did not investigate the direct relationship between total flavonoid content and the pharmacological activities of tempuyung leaves. Therefore, future studies should focus on the isolation and identification of specific flavonoid compounds, for example, using chromatographic or spectrometric techniques, followed by the evaluation of the biological activities of fractions exhibiting high total flavonoid content.

CONCLUSION

The determination of total flavonoid content revealed differences among the fractions, with the aqueous fraction containing 17.111 mg QE/g (1.7111%), the ethyl acetate fraction containing 91.887 mg QE/g (9.1887%), and the n-hexane fraction containing 79.333 mg QE/g (7.9333%). Thus, the ethyl acetate fraction exhibited the highest total flavonoid content, followed by the n-hexane and aqueous fractions. These findings indicate that the

flavonoids quantified in tempuyung leaves were more abundantly distributed in the semipolar fraction (ethyl acetate) than in the polar and nonpolar fractions. Overall, the findings demonstrate that fractionation based on differences in solvent polarity can provide more specific information regarding the distribution of flavonoids in tempuyung leaf extract.

The findings of this study provide scientific implications by offering information on the distribution of total flavonoid content across different levels of solvent polarity, with the ethyl acetate fraction exhibiting the highest total flavonoid content. These findings may serve as preliminary data for further research, particularly for the purification, isolation, and identification of specific flavonoid compounds present in the ethyl acetate fraction. This is consistent with the limitation of the present study, which was restricted to the determination of total flavonoid content; therefore, further purification is required to identify the specific types of flavonoids present in the samples.

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